Optimization of 3D printed hydrogels with primary cells for tissue engineering

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Abstract

3D bioprinting, a flexible automated on demand platform for free form fabrication of complex living architectures, is a novel approach for design and engineering of human organs and tissues (1). We here report on a tissue engineered Anterior Cruciate Ligament (ACL) scaffold fabricated using 3D bioprinting technology. The objective of this *in vitro* study was to optimize the printing parameters for maximum cell viability, determinate cellular response and characterize mechanical properties of materials. Here, human ACL cells were used as constituent cells and mixture of gelatin, fibrinogen and hyaluronic acid were used as cell laden hydrogel. Cell localization and proliferation were investigated to study cell functions and tissue formation process in 3D structure.

Introduction

The anterior cruciate ligament is most commonly injured ligament of knee (2). ACL does not heal due to poor healing potential and limited vascularization. Therefore, surgical invervention is usually used and several ACL reconstruction surgeries are performed such as autografts, allografts and synthetic grafts. However, the long term clinical outcome of these grafts was suboptimal due to poor abrasion resistance and limited integration between grafts and host tissues. These outcomes have prompted a growing interest in tissue engineering solutions for ACL reconstructions. Our approach is to demonstrate the potential of 3D bioprinting for ACL constructs.
The aim of this study was to optimize in vitro 3D bioprinted scaffold design for ligament tissue engineering, focusing on material selection and printing parameters based on cellular response and mechanical properties of materials.

Material and Methods

ACL scaffolds were produced using RegenHU bioprinter equipped with 2 different print heads. The diameter of scaffolds is 3 mm and the height of scaffolds is 0.5 mm. Bioink solution includes gelatin (35 mg/ml), fibrinogen (25 mg/ml) and hyaluronic acid (3 mg/ml). Transglutaminase and thrombin used as crosslinking agents. Bioink solution and crosslikers were printed from different printing heads. The bioink was mixed 500 µl of ACL cells ($1x10^6$ - $4x10^6$ per ml) and the cell laden ink was printed by 150 µl nozzles. As a control, the hydrogel was printed and ACL cells were seeded on the printed structures to test bioprinting process effect on cell viability. The viability of

printed cells were examined using calcein-ethidium homodimer dyes under the LSM710 confocal microscopy for 4 different time points.

Results

The printed constructs were cultured for 21 days. The viability of ACL cells was analyzed on Day1, Day 3, Day 7 and Day 14. High cell viability was observed for both the seeded and printed ACL cells group. After 7 days, seeding cells began to fill in the printed pattern.

Discussion

In this work, we describe the development, optimization of a 3D bioprinted ACL scaffold and determinate an appropriate printing parameter with respect to cellular response and mechanical properties. To this end, this study examined the effect of printing process and the attachment and proliferation of primary human ACL cells. The results showed that the viability of cells was not affected by printing process. Future work will involve also the usage of primary intervertebral disc cells in order to test applications on chip.

References

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